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AN EVALUATION OF ADENOSINE 3′,5′-CYCLIC MONOPHOSPHATE-DEPENDENT PROTEIN KINASE ACTIVITY IN ATOPIC DERMATITIS*

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Activity of adenosine 3′,5′-cyclic monophosphate-dependent protein kinase has been measured in the skin of normal controls, patients with non-atopic skin disorders, and those with atopic dermatitis. All samples analyzed displayed the presence of this enzymatic activity. However, the enzyme from the atopic skin did not seem to be dependent on cyclic AMP for activity. Whether this is due to an artifact of isolation of protein kinase or is indeed the true in vivo nature of the enzyme remains to be established.

According to Szentivanyi [1], atopy is an adrenergic imbalance caused by an increase in alpha adrenergic activity and a decrease in beta adrenergic activity or both. Although only the asthmatic manifestations of atopy were his main considerations, Szentivanyi conceded that his theory could apply equally well to other clinical expressions of atopy. This concept considers the cyclic AMP system as the probable site of the biochemical lesion in atopy (atopic dermatitis).

In support of this theory, asthmatics exhibit less increase in urinary cyclic AMP in comparison to normal subjects when challenged by epinephrine [2,3]. Asthmatics also display a malfunction in platelet aggregation after stimulation by epinephrine, suggesting altered adrenergic receptors in their platelets [4]. Similarly, leukocytes of patients with atopic eczema or asthma manifest a lowered response to beta adrenergic stimulation by isoproterenol [5,7]. Previous studies resulting in detection of lower levels of catecholamines in the plasma [8] and their altered disposition in the skin of patients with atopic dermatitis [9] also sustain the postulates later suggested by Szentivanyi [1].

On the other hand, normal activities of adenylate cyclase [10], cyclic AMP phosphodiesterase [11], and cyclic AMP-dependent protein kinase [12] have been found in skin biopsies from patients with atopic dermatitis.

In this communication, we report the results of our investigations on cyclic AMP-dependent protein kinase activity in the skin of normal subjects, and that of patients with non-atopic skin disorders and atopic dermatitis. In contrast to the report of Mier and co-workers [12], we observed that protein kinase of atopic skin exhibited relatively small or no stimulation of enzyme activity in the presence of cyclic AMP when compared to the two types of controls we employed. This observation suggests the presence of an altered enzymatic entity or entities in the skin of patients with atopic dermatitis.

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*The abbreviations used are: cyclic AMP, adenosine 3′,5′-cyclic monophosphate; cyclic GMP, guanosine 3′,5′-cyclic monophosphate; cyclic CMP, cytidine 3′,5′-cyclic monophosphate; cyclic UMP, uridine 3′,5′-cyclic monophosphate.

MATERIALS AND METHODS

Punch biopsies (4-mm) of skin from non-atopic and atopic patients were acquired from the Dermatology Clinic of the University Hospital. Informed consent was secured from each patient. Freshly excised clinically normal skin was obtained from the operating room.

γ-[^{32}P]ATP and ^{3}H-labeled cyclic AMP were purchased from New England Nuclear Corporation. Histone, cyclic AMP, and the other cyclic nucleotides were the products of Sigma Chemical Company or P-L Biochemicals.

Assays for protein kinase activity and cyclic AMP-binding activity were performed as described in detail previously [13,14]. Specific activity of the enzyme was expressed as picomoles of ^{32}P incorporated into histone by 1.0 mg of enzyme per 10 min incubation at 37°C. Protein concentration was determined by the method of Lowry et al [15] using bovine serum albumin as the standard.

Enzyme purification. All operations were performed at 2–4°C. Skin cleaned of subcutaneous tissue was pulverized in a steel mortar and pestle cooled in liquid nitrogen. The powder was suspended in 0.5 ml of cold 0.02 M Tris–HCl, pH 7.5, containing 1 mM dithithreitol (Buffer A) and sonicated for 5 sec. The resultant solution was centrifuged at 105,000 × *g* for 90 min and the supernatant treated as previously reported [13,14], successively with 1% streptomycin sulfate and 50% ammonium sulfate to isolate the enzyme. Protein kinase and cyclic AMP binding activities as well as protein concentration were determined on this preparation after exhaustive dialysis against Buffer A.

RESULTS AND DISCUSSION

Figure 1 summarizes the protein kinase activity in the skin of normal, non-atopic, and atopic

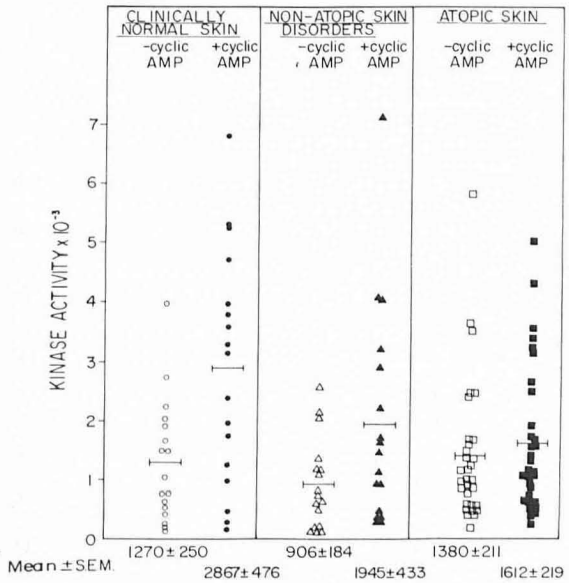

FIG. 1. Cyclic AMP-dependent protein kinase activity in the skin of normal, non-atopic, and atopic subjects. Biopsies of non-atopic and atopic patients were taken from active lesions. The enzyme activity was measured as described previously [13, 14]. The average enzyme concentration in the assay was 0.61 mg per ml of normal, 0.54 mg per ml of non-atopic, and 0.13 mg per ml of atopic preparation. Kinase activity is expressed as picomoles of ^{32}P incorporated into histone by 1.0 mg of enzyme per 10-min incubation at 37°C. Specific activity of γ-[^{32}P] ATP was 22–25 cpm per pmole. The non-atopic skin originated from the following clinical conditions: scar tissue, psoriasis, carcinoma of the breast, ichthyosis, cystoma, fixed drug eruption, mycosis fungoides, exfoliative dermatitis, junctional nevus, Kaposi's sarcoma, contact dermatitis, vasculitis, scleroderma, dermatofibroma, melanoma, and uninvolved skin from atopic dermatitis.

patients. It is evident that the protein kinase activity in individual samples within each group varied over a wide range and all tissues examined exhibited a finite amount of enzyme activity both in the presence and in the absence of cyclic AMP. The most significant observation however, was that, unlike the enzyme from normal and non-atopic controls, the enzyme from atopic skin consistently displayed little or no stimulation of activity in the presence of cyclic AMP. As indicated in Figure 2, the percent stimulation of enzymatic activity in the presence of cyclic AMP was, for normals, 130 ± 13 (mean ± SEM); for non-atopics, 115 ± 11; and for atopics, 29 ± 6. By the *t*-test of Student, the differences between the controls and atopics are significant ($p < 0.001$). As shown here, cyclic AMP at 1000-fold higher concentration (5×10^{-3} M) also did not elicit a stimulatory response of protein kinase activity in atopic skin. Therefore, the presence in atopic patients of a cyclic AMP-dependent protein kinase(s) with modified K_m for cyclic AMP seems unlikely. Replacement of cyclic AMP by cyclic GMP, cyclic UMP, or cyclic CMP at concentrations of 5×10^{-6} M was also without effect (Fig. 2), suggesting that the kinase in atopic skin did not have an altered specificity with respect to its cyclic nucleotide requirement. Furthermore, of the 10 samples of enzymes from the atopic skin tested for cyclic AMP binding, only one displayed any measurable amount of [^{3}H]cyclic AMP binding (2.9 picomoles/mg protein). Protein kinases of normal skin [14] under similar conditions bound at least 10 to 20 picomoles of [^{3}H]cyclic AMP per mg protein depending upon the preparation (data not shown).

The results reported here deviate from those of Mier et al [12] with regard to the effect of cyclic AMP on protein kinases from atopic skin. Whether this is a result of the assay conditions or is due to some other unrecognized factors is not clear to us.

It is well known that cyclic AMP-dependent protein phosphotransferases normally exist as inactive oligomers constructed from two types of subunits: catalytic and regulatory [16]. Cyclic AMP dissociates and activates the enzyme by binding to the regulatory subunit and releasing the catalytic moiety to perform its function [16]. The data presented here seem to suggest that cyclic AMP is ineffective in stimulating the protein phosphotransferases of atopic skin, probably due to an apparent absence of the cyclic AMP-binding regulatory subunit in these enzymes. However, it is conceivable that the lack of stimulation by cyclic AMP is an artifact of isolation of the enzyme from small amounts of tissue resulting in the dissociated species. Our observations also do not specify whether one or all of the multiple forms of cyclic AMP-dependent kinases normally present in skin [14] has been modified in the atopic skin. The effect of the heat-stable inhibitor of protein kinase

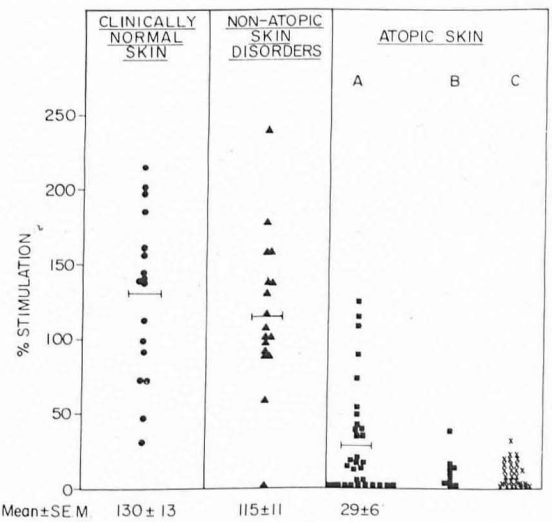

FIG. 2. Percent stimulation of protein kinase activity. For normal and non-atopic skin, the stimulation denoted is in the presence of 5×10^{-6} M cyclic AMP while for atopic skin it is in the presence of: (*A*) 5×10^{-6} M cyclic AMP; (*B*) 5×10^{-3} M cyclic AMP; (*C*) 5×10^{-6} M cyclic GMP or cyclic UMP or cyclic CMP.

[16] as well as that of cyclic AMP-binding regulatory subunits isolated from normal skin protein kinase on the enzyme from atopic skin should enable us to resolve these questions.

Relatively larger amounts of normal and nonatopic skin have been employed in the extraction of protein kinase resulting in enzyme concentrations in the range of 0.7 to 4.9 mg per ml and 0.2 to 5.8 mg per ml, respectively. In contrast, the atopic biopsies yielded less tissue as the enzyme source and therefore lower protein concentrations of 0.2 to 2.0 mg per ml. Whether this difference in protein concentration has any bearing on our results also has to be ascertained.

Since both cyclic AMP-dependent and -independent protein kinases have been identified in the same tissue [17], the atopic cells may have been programmed to synthesize one kind exclusively or the regulatory subunit of one may be nonfunctional. A preferential loss of the cyclic AMP-binding regulatory proteins during purification or the presence of an as yet unknown inhibitor of the binding proteins in the atopic cells are other possibilities. A definitive elucidation of these alternatives must await the isolation and purification of the various isozymes from atopic skin in large enough quantities for further analysis. In this context, it is worthwhile to draw attention to the reports on the identification and isolation of certain mouse lymphoma and rat hepatoma cells which are deficient in cyclic AMP-binding proteins [18–20]. More recently, mutations affecting one or both types of subunits of cyclic AMP-dependent protein kinase in mouse lymphoma cells also have been detected [21].

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